by specific environmental conditions. Thus, in the conversion of fibrinogen to fibrin, it seems likely that the loss of highly charged fibrinopeptides (*4*) alters the interaction properties of the building units without causing profound changes in internal structure.

Preliminary observations indicate that fibrin stained with uranyl acetate (*5*) shows a density distribution in the electron microscope similar to that reported here for stained fibrinogen tactoids (*6*).

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References and Notes

1. L. Stryer, C. Cohen, R. Langridge, *Nature* **197**, 793 (1963).
2. K. Laki, *Studies Inst. Med. Chem., Univ. Szeged* (1942).
3. D. Bagdy, *Hung. Acta Physiol.* (1948 p. 18.
4. F. R. Bethelheim and K. Bailey, *Biochim. Biophys. Acta* **9**, 578 (1962); K. Laki, J. A. Gladner, J. E. Falk, *Nature* **187**, 758 (1960); B. Blomback and J. Sjoquist, *Acta Chem. Scand.* **14**, 493 (1960).
5. After discussion with Drs. C. Hall and H. Slayter.
6. Supported in part by U.S. Public Health Service grants A-2633 (C.C.) and SG-13,979 (J-P.R.) from the National Institutes of Health. We thank Professor R. S. Bear, and Drs. D. L. D. Caspar and R. Langridge for useful discussions.

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Elastic Membrane: Effect of Increasing Tension on the Absorptive Capacity

Arterial walls contain a large proportion of elastin, a tissue polymer with long-range elasticity similar to that of rubber. In arterial hypertension the arterial walls are under constantly elevated tension. In atherosclerosis, a disease considered to be enhanced by arterial hypertension (*1*), adsorption of blood lipids to and into the arterial wall is thought to be an important pathogenic factor (*2*). Moreover, it has been shown, in electron-microscopic studies, that one of the earliest steps in the pathogenesis of experimental atherosclerosis is the inhibition of lipid by the internal elastic lamina (*3*). Therefore it seems reasonable that enhancing of adsorptive capacity of the internal elastic lamina may be one of the means by which hypertension predisposes to atherosclerosis.

Before exploring this idea, it would be desirable to know whether increasing the tension of elastic substances in general would increase their ability to adsorb molecules from solution. Experiments were conducted with models of heavy latex rubber dam (*4*) to simulate the elastic adsorbent membrane; Evans blue was used for the substance to be adsorbed.

A 0.3 percent solution of Evans blue in McIlvaine's buffer with *p*H of 4.8 was prepared. Pyrex test tubes with lips having an outside diameter of 2.8 cm and with their closed ends cut out were used as receptacles for the dye solution. Circles with diameters of 2.2, 2.4, 2.6, and 2.8 cm were traced in black on sheets of heavy latex-rubber dam with a ball-point pen. Each sheet, with the drawn circle on the outer surface, was stretched over the lipped end of the test tube so that the inked line of the circle coincided with the outer border of the lip, and was fixed in position by rubber bands wound tightly around the dam and the tube just below the lip. After the dam had been stretched and fixed to the tube, the traced circle was always 2.8 cm in diameter. Therefore the ratios of the stretched to the unstretched diameters of the various sized circles were: 2.8/2.8, 2.8/2.6, 2.8/2.4, and 2.8/2.2. Two milliliters of dye solution was added to each tube covered by dam and left there for 6 hours. The inside of the tube and the inner latex surface were rinsed thoroughly with distilled water and 3 ml of distilled water was left in each tube for 15 minutes. After the water was completely drained from the tube, the dye adherent to the dam was eluted by leaving 3 ml of 1 percent aqueous solution of sodium carbonate in the tube for exactly 1 hour. The eluate was then poured into a cuvette and its optical density read at 580 mμ. The optical density was converted to micrograms of dye by a regression equation obtained by plotting the optical densities of several dye solutions of known and varying concentration. The data were expressed as micrograms of dye per square centimeter of stretched membrane for each "area-stretch" ratio (the area of the stretched circle on the membrane divided by the area of circle before stretching).

There was significant increase in adsorptive capacity ($p < .01$) by the stretched membrane with increasing tension up to an area-stretch ratio of 1.36 (Table 1). Also, up to this point the increase was found by semilogarithmic plot to be exponential ($y = 3.67e^{.577}$). Beyond a stretch ratio of 1.36 increase in adsorptive capacity of the stretched membrane ceased. Although the shape of the experimental curve plotted from the data in the table seems to suggest an equilibrium, it is not clear whether this phenomenon involves equilibria or rates of adsorption and diffusion in the membrane.

Table 1. Effect of increasing tension on the capacity of latex rubber dam to adsorb Evans blue.

Area stretch ratio	Trials (No.)	Mean quantity of dye adsorbed by stretched dam (μg/cm^2)	Standard error
1.00	14	5.22	.10
1.16	17	6.42	.08
1.36	18	8.32	.05
1.62	16	8.58	.14

The reason for the increase of adsorptive capacity of the rubber membrane with increasing tension may possibly be found in the explanation of the phenomenon of elasticity of rubber-like substances (*5*).

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References and Notes

1. I. H. Page, *Circulation* **10**, 1 (1954).
2. G. L. Duff and G. C. McMillan, *Am. J. Med.* **11**, 9 (1951); S. L. Wilens, *Science* **114**, 389 (1951).
3. J. Parker, *Am. J. Pathol.* **36**, 19 (1960).
4. Obtained from Hygienic Dental Manufacturing Company, Akron 10, Ohio.
5. P. J. Flory, *Principles of Polymer Chemistry* (Cornell University Press, Ithaca, 1953); M. L. Huggins, *Physical Chemistry of High Polymers* (Wiley, New York, 1958).

29 April 1963

Renal Tubular Localization of Chlormerodrin Labeled with Mercury-203 by Autoradiography

Abstract. *An autoradiographic technique has been developed for determining the intrarenal distribution of therapeutic doses of chlormerodrin labeled with mercury-203. Highest concentrations of mercury were detected in the straight portion of the proximal tubule in the rat and in the convoluted portion of the proximal tubule in the dog.*

It is generally accepted that mercurial diuretics act by inhibiting renal reabsorption of sodium and water, although the mechanisms of action and tubular site at which this effect occurs remain controversial. Conventional studies of renal function and histochemical methods have failed to resolve these questions (*1, 2*). If it is assumed

that deposition of mercury in the tubules is a prerequisite for pharmacologic activity, anatomic location of mercury might aid in identifying the site of diuretic action. However, the site of diuretic activity need not be identical with that of maximum concentration of mercury. An autoradiographic technique has been developed in this laboratory for the study of the distribution of mercury compounds in renal tissue. This method permits identification of the separate nephron sites containing radiomercury (Hg^{203}) and a semiquantitative appraisal of mercury concentration.

The tubular localization of a therapeutic dose of Hg^{203}-chlormerodrin (*3*) was determined in dogs and rats. In dogs, to insure diuresis, 6.0 g of ammonium chloride was administered orally for 2 days before each experiment. Bilateral ureteral catheterization was performed in anesthetized dogs, and the rate of urine flow and chloride excretion was determined for each kidney.

Renal biopsy or nephrectomy was performed 15 to 120 minutes after the intravenous administration of the therapeutic dose of Hg^{203}-chlormerodrin (1.0 mg, or about 200 μc, of mercury per kilogram of body weight). Similar studies were made with subdiuretic doses which contained 0.1 mg of mercury per kilogram. Rats were treated only with the therapeutic dose and were killed 6 hours after a single intraperitoneal dose.

The tissues were fixed in 10 percent neutral Formalin, embedded in paraffin, sectioned to a thickness of 5 μ, and mounted on glass slides. Less than 10 percent of the Hg^{203} was lost from the tissues during the processing. Autoradiographs were obtained by dipping the glass slides into Kodak liquid NTB2 emulsion, exposing the film for 10 days and developing in a 20:1 dilution of D19 developer. The unstained preparation was dehydrated in graded alcohols and xylol, mounted in oil (1.530 refractive index), and covered with a cover slip. Preliminary identification of tubular structures was made by phase microscopy. Distribution of developed silver grains in the autoradiograph was examined by transmitted-light microscopy. Slides were subsequently stained by the periodic-acid-Schiff (PAS) method. The stain removed the visible silver grains from the emulsion. Since the brush border of the proximal tubule stains red by this method, differentiation of proximal from distal tubules was facilitated. Comparison of photomicrographs of the same field of the autoradiograph and the stained tissue permitted tubular localization of Hg^{203}.

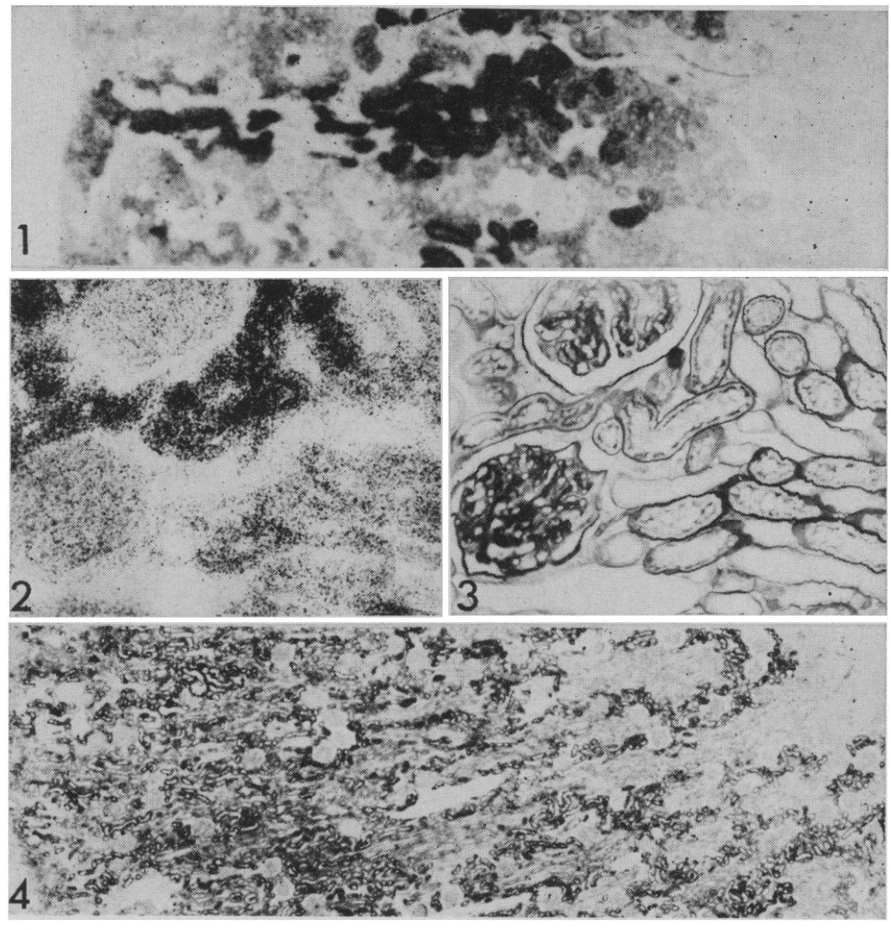

Fig. 1. Autoradiograph of Hg^{203}-chlormerodrin in the rat kidney. Nephrectomy was performed 6 hours after intraperitoneal administration of a therapeutic dose. Unstained; outer cortex on left, outer medulla on right (about × 55). Fig. 2. Autoradiograph of Hg^{203}-chlormerodrin in the dog kidney. Biopsy was taken 90 minutes after intravenous administration of a therapeutic dose. Unstained; inner cortex (about × 250). Fig. 3. Same field as Fig. 2, stained by PAS. Proximal tubules show PAS-positive brush borders. Highest grain density corresponds to proximal tubules surrounding glomeruli (about × 250). Fig. 4. Autoradiograph of the kidney shown in Fig. 2, unstained; outer cortex on left, outer medulla on right (about × 15).

The distribution of mercury shown by autoradiography differed in the dog and rat kidney. In the rat, maximum grain density was observed over the straight portion of the proximal tubule (Fig. 1). In contrast, the dog kidney showed maximum grain density over the convoluted portion of the proximal tubule (Figs. 2–4). In both species the radioactivity over distal tubules and glomeruli was low, and radioactivity in the papilla approximated that of the background. In the dog the distribution of mercury in the tissues from the 15-minute biopsies and after the subdiuretic dose of Hg^{203}-chlormerodrin was the same as the distribution when diuresis was at its peak.

Our autoradiographic technique demonstrates a difference in the tubular distribution of therapeutic doses of mercury in rats and dogs. This finding is consistent with the species variation in the renal metabolism of Hg^{203}-chlormerodrin (*4*). However, this species difference has not been noted in studies where other histochemical techniques were used. Reduction of sulfhydryl staining and necrosis of cells after the administration of mercury has been observed primarily in the straight portion of the proximal tubule in both the rat and the dog (*2*).

Although the concentration of mercury was greater in proximal than in distal tubular segments in both species, this distribution does not necessarily indicate the site of diuretic activity. It is conceivable that the high concentration in proximal tubule cells reflects tubular secretion at this site rather than diuretic action (*5*).

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References and Notes

1. M. F. Levitt and M. H. Goldstein, *Bull. N.Y. Acad. Med.* **38**, 249 (1962); F. Timm and M. Arnold, *Arch. Exptl. Pathol. Pharmakol.* **239**, 393 (1960).
2. A. Farah, C. H. Bender, R. Kruse, E. Cafruny, *J. Pharmacol. Exptl. Therap.* **125**, 309 (1959); J. G. Edwards, *Am. J. Pathol.* **18**, 1011 (1942); J. P. Simmonds and O. E. Hepler, *Arch. Pathol.* **39**, 103 (1945).
3. Radio-Neohydrin, Abbott Laboratories, Oak Ridge, Tenn.
4. R. R. M. Borghgraef and R. F. Pitts, *J. Clin. Invest.* **35**, 31 (1956).

5. Supported by a training grant (HTS-5505) from the National Institutes of Health.

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Control and Training of Individual Motor Units

Abstract. *Experiments clearly demonstrate that with the help of auditory and visual cues man can single out motor units and control their isolated contractions. Experiments on the training of this control, interpreted as the training of descending pathways to single anterior horn cells, provide a new glimpse of the fineness of conscious motor controls. After training, subjects can recall into activity different single motor units by an effort of will while inhibiting the activity of neighbors. Some learn such exquisite control that they soon can produce rhythms of contraction in one unit, imitating drum rolls, etc. The quality of control over individual anterior horn cells may determine rates of learning.*

It is a commonplace observation that very gentle contractions of skeletal muscles recruit only a few motor units and that, on relaxation, human beings can promptly repress all neuromuscular activity in large areas under voluntary control (*1*). However, little attention has been paid to the fine voluntary control of individual motor units. In 1960 Harrison and Mortensen (*2*) reported that subjects were able to maintain isolated activity of several different motor units in the tibialis anterior as recorded from surface electrodes and confirmed by needle electrodes. The implications of this finding led to an intensive systematic investigation with special indwelling electrodes.

By definition, a motor unit includes a spinal anterior horn cell, its axon, and all the muscle fibers on which the terminal branches of the axon end (Fig. 1). This motor unit "fires" when an impulse reaches the muscle fibers, the response being a brief twitch. The electrical potential accompanying the twitch is now well documented. The twitch frequency has an upper limit of about 50 per second. With indwelling electrodes, individual motor units are identifiable by their individual shapes; these remain relatively constant unless the electrodes are shifted.

The subjects of these experiments were provided with two modalities of "proprioception" that they normally lack, namely, they heard their motor unit potentials and saw them on monitors. The subjects were 16 normal persons ranging in age from 20 to 55. All but five were under 24 and only one was female.

The main muscle tested in all subjects was the right abductor pollicis brevis (Fig. 2). In two subjects the tibialis anterior was also tested; in another, the biceps brachii and the extensor digitorum longus were tested on other occasions. The recording and monitoring apparatus is illustrated in Fig. 2.

The indwelling electrodes used have already been described in detail (*3*). They are nylon-insulated Karma alloy wires 0.025 mm in diameter, which are introduced into the muscle as a pair by means of a hypodermic needle that is immediately withdrawn. In the case of a small muscle like the abductor pollicis brevis, the activity of all its motor units are probably detected while the fascial coat of the muscle isolates the pick-up to this muscle alone.

After placement and connection of the electrodes, the subjects spent 5 to 10 minutes becoming familiar with the response of the electromyograph to a range of movements and postures. They were invariably amazed at the responsiveness to even the slightest effort. Then they began learning how to maintain very slight contractions, which were apparent to themselves only through the response of the apparatus. This led to increasingly more demanding effort involving many procedures intended to reveal both their natural talent in controlling individual motor units and their skill in learning and retaining tricks with such units. Individual units were identified by the characteristics of their potentials which show considerable difference on the oscilloscope and, to a lesser extent, on the loudspeaker. Film recordings of potentials were made for confirmation (Fig. 3).

Generally, experiments on one muscle were limited to about half a day. Within 15 to 30 minutes all subjects had achieved notably better willful control over gentle contractions. In this

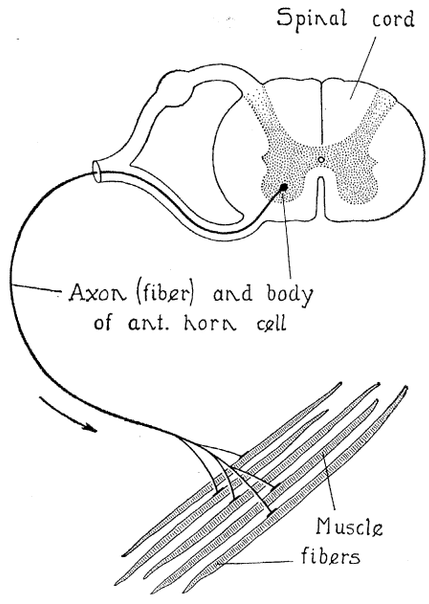

Fig. 1. Diagram of a motor unit of skeletal muscle.

time almost all had learned to relax the whole muscle instantaneously on command and to recruit the activity of a single motor unit, keeping it active for as many minutes as desired. A few had difficulty maintaining the activity of such a unit, or in recruiting more units. No relationship was obvious to age, manual dexterity, or anything that might have been invoked as an underlying explanation of the differences in performance. Two of the apparently most dexterous persons performed only moderately well. The youngest persons were among both the worst and the best performers. General personality traits did not seem to matter.

After about 30 minutes the subject was required to learn how to repress the first motor unit he had become familiar with and to recruit another one. Most subjects were able to do this and gain mastery of the new unit in a matter of minutes; only one subject required more than 15 minutes. More than half of the subjects could repeat the performance with a third new unit within a few minutes. A few subjects could recruit a fourth or a fifth isolated unit. The next problem facing a subject was to recruit, unerringly and in isolation, the several units over which he had gained control.

Here there was a considerable variation in skill. About one in four could respond easily to the command for isolated contractions of any of three units. About half the subjects displayed much less skill in this regard, even after several hours and even though they